# Implication and clinical application of translational medicine in the management of common urologic cancers

*by*

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# Contents

# Abstract

The eight peer reviewed publications and 11 posters/abstracts of presentations which form the basis of this PhD, investigate the implementation of novel diagnostic tools and the application of these findings to identify possible therapeutic targets for management of varying stages of disease progression in common urologic cancers. The publications are drawn predominantly from basic urologic cancer research.

**Chapter One** introduces the concept of translational medicine in urologic cancer research. Although clinical application is an integral part of urologic practice, medicine remains a scientific discipline and research forms an essential component thereof. The genomic profiling of multiple urologic cancers has allowed a more in depth appreciation into the intricacies of the disease. Investigating the genomic landscapes of urologic cancers has helped to identify driver gene alterations and their clinical implication on affected patients. Better understanding of disease development and progression may guide clinical decision-making, particularly in view of recent biomarker discoveries and targeted drug development for a more precision based approach to the oncological management of these patients.

**Chapter Two** presents the implementation of translational tools in the uro-oncologic setting. Practical and financial challenges of routine collection and utilisation of research results in clinical practice remains a distant goal. Nonetheless the studies presented in this chapter explore the use of the basic research and the applicability of the results obtained.

**Chapter Three** summarizes the discussion on the treatment-based application of translational medicine in urologic cancers. The aim to strive for a more comprehensive understanding of the urologic diseases we manage will guide precision treatment approaches to address the oncological needs of individual patients.

# Declaration

Whilst registered as a candidate for the above degree, I have not been registered for any other research award. The results and conclusions embodied in this thesis are the work of the named candidate and have not been submitted for any other academic award.

# Acknowledgements

This thesis represents a selection of my academic work over the last decade. I am eternally grateful to mentors and colleagues alike who have driven me to be investigative, in particular: Professor Klaus-Peter Dieckmann who sparked my scientific interest and Dr. Alexander Wyatt who identified my potential and set the bar of expectation I strive toward. Dr. John Young, for taking me on as a student, for his advice and enthusiasm toward this project. My parents, Louise and Joachim, for their relentless encouragement.

Finally to all the patients and their families for allowing us to pursue our goals with their best interests at heart to ever improve our care for them.

# Preface

Translational medicine in uro-oncology strives to understand the dynamics of cancer development, progression and treatment response in a bench to bedside approach. As part of this clinical application is essential in identifying treatment modalities that allow for individualised cancer care. The narrative of this thesis combines eight of the published peer reviewed articles and four of the abstracts that I have authored or co-authored over a 10-year period. These articles are included in their full text format in the appendices.

## *Curriculum vitae*

I have been working as a specialist urologist in a clinical setting since completion of my training in Hamburg, Germany, in 2014. During our training, emphasis was mainly placed on the clinical aspects necessary to ensure a clear understanding of diagnosis, clinical management (conservative or interventional) and follow-up of urologic disorders to enable secure, independent decision-making for optimal patient care. Although clinical application is an integral part of urologic practice, medicine remains a scientific discipline and research forms an essential component thereof.

As a trainee I was intrigued by research investigations undertaken by my peers and followed suit completing a prospective clinical study investigating the immediate vascular impact of cisplatin-based chemotherapy on young patients diagnosed with testicular cancer. As my interest migrated toward uro-oncology during the latter part of my specialist training, I applied for a onco-urology Fellowship in Vancouver, Canada. The fellowship was research based (with clinical duties) and I was able to nurture an interest in the genomic profiling of prostate and bladder cancer. Being part of a rising genomics laboratory team was a privilege. Investigating the genomic landscapes of these diseases helped to identify driver gene alterations and their clinical implication on affected patients. We were able to publish impactful peer reviewed studies to this extent. This work reiterated my desire to work as a clinician-scientist, with the intent to better understand disease development and progression which may guide clinical decision-making, particularly in view of recent biomarker discoveries and targeted drug development for a more precision based approach to oncological management of these patients.

I am currently working in the Department of Urology, at the University Hospital Southampton, with a particular interest in prostate and bladder cancers. The department creates a supportive environment for focused skills' development and I believe our interests for research-based initiatives are ever growing, to address the needs and requirements of our patients. Ultimately a more comprehensive understanding of the diseases we manage will allow for greater individualised treatment approaches to address the oncological needs of our patients.

# Chapter 1: translational medicine in urologic cancer

## 1.1 Introduction

In the vastly progressive field of biomedicine an interdisciplinary branch of translational medicine (TM) has recently emerged. The term, first coined in the 1990's, historically emphasized the concept of translating laboratory discoveries into practical clinical applications that would benefit the patient.[1] Despite this fundamental step, the initial concept focused on *unilateral* benchside expertise driving clinical application, thereby missing crucial bedside feedback. This demonstrated clear limitations. Overall advancement in biomedical research became increasingly dependent on multi-disciplinary groups. The involvement of biomedical, clinical and basic scientists together with engineers and emerging technologies led to the evolution of a reciprocal appreciation for the benchside and bedside concept.[2] Returning clinical findings to research laboratories may redefine or enable new hypothesis-driven research efforts, resulting in potentially innovative discoveries.

Potential pitfalls impacting on traditional bedside-to-benchside pathways present themselves when promising benchside discoveries fail to provide any significant bedside outcome. Notwithstanding, this two-way concept still misses an important aspect of the healthcare cycle: the community.[3] The community represents healthy populations and/or patients as well as medical practitioners. All are vital to TM. Besides enriching TM with valuable input regarding background information on general health thereby enhancing existing tools and treatments, the community can promote involvement of patients groups and healthy volunteers in clinical trials. Finally, community involvement may also provide alternate sources of funding through grants, endowments and general fundraising activities. Financial expenditure often hampers research development particularly in social healthcare systems as funding is often limited to good basic healthcare coverage. Thus generating income outside of these structures is essential.

The European Society for Translational Medicine (EUSTM) has therefore defined translational medicine as an interdisciplinary branch of the biomedical field supported by three main pillars: benchside, bedside and community. The goal of TM is to

combine disciplines, resources, expertise, and techniques within these pillars to promote enhancements in prevention, diagnosis, and therapies.[3]

### 1.2 Use of translational medicine in urology

Translational medicine in uro-oncologic disease research is focused on understanding underlying mechanisms of disease development and progression. The identification of new cancer pathways and their related interactions provides valuable information about disease evolution. Tools to identify patients with potential predisposition for early onset of urologic cancers may aid earlier surveillance or treatment with improved chances of cure. Furthermore, translational medicine offers a potential opportunity to research and identify processes that can stifle disease progression and action advanced disease on an individualised basis. The benefit of implementing newer and more innovative approaches to current treatment plans may improve patient outcomes and quality of life.

A three-way approach to uro-oncological research focuses heavily on developing, advancing and implementing novel therapeutics and treatment paradigms in cancer patients. Clinical application gives clinician-scientists insights to response and new avenues to explore, driving renewed benchside development. Community-based application in the form of clinical trials aid in assessing expectations.

### 1.3 Aims of this thesis

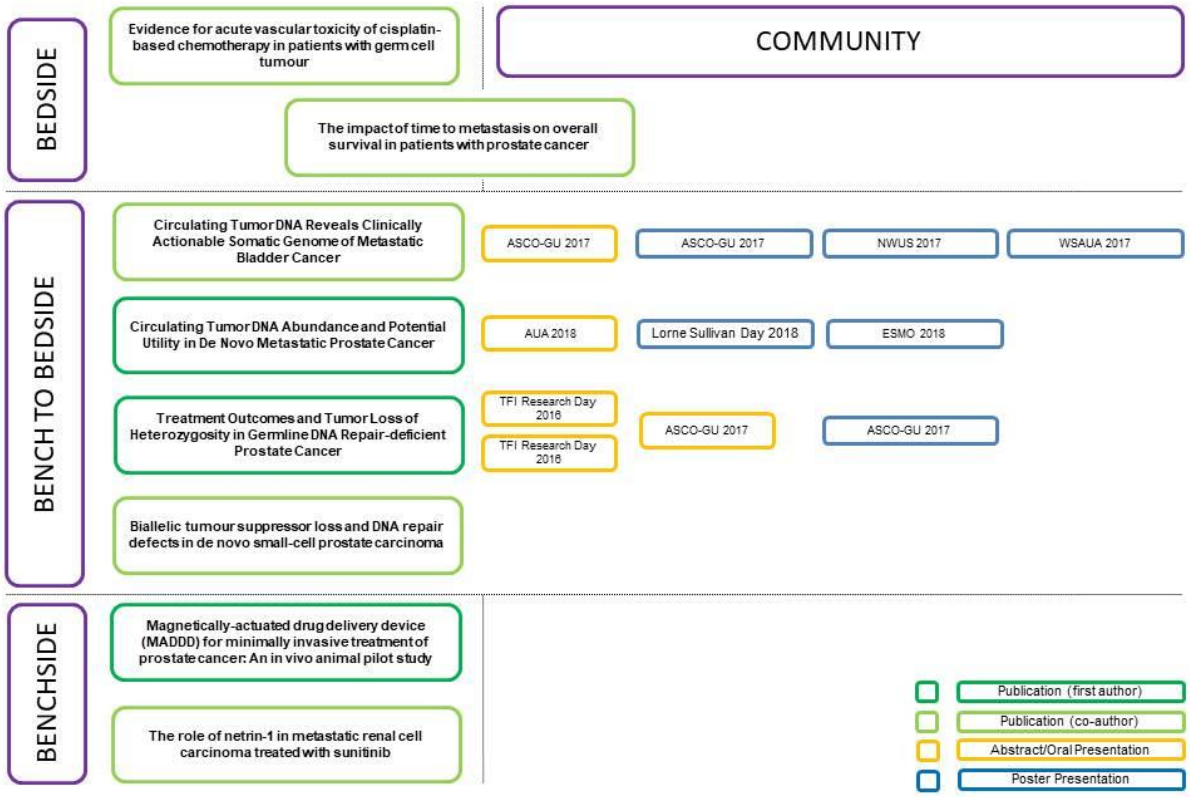
This thesis presents several studies that demonstrate the diverse application of novel research-based tools in common urologic cancers. The application of these tools has improved our understanding of the dynamics of urologic cancers. As a result insight is gained into the clinical implications and invoke consideration for therapies that more readily address the causes than conventional treatment options may do alone.

The studies featured in this manuscript are wide-ranging and besides focusing on localised and treatment naive metastatic disease, encompass metastatic treatment-resistant urologic cancers as well. The translational tools implemented are diverse;

spanning medical devices, clinical impact assessments to minimally invasive blood tests investigating the genomic composition of cancer DNA as well as establishing surrogate blood markers to identify potential cancer patients at risk of conventional treatment complications.

This thesis aims to emphasize the core principles of translational medicine in urologic cancer. Benchside discoveries aim to promote bedside application. The reimplementation of these discoveries expedite renewed studies to further develop tailored oncological management.

Figure 1: Studies integrated into this thesis illustrating the diverse application of novel research tools in common urologic cancers



## Chapter 2: Implementation of translational tools in uro-oncology

During the past three decades, mortality has fallen substantially in an aging population (in high-income countries). This decrease is due to a historic change in the development of worldwide health status.[4] Despite general improvements in health care, the incidence and mortality of urologic cancers, in particular those of bladder, prostate and kidney, vary significantly across the globe.[5,6,7] Alarmingly the cumulative incidence of kidney, bladder, and prostate cancers has risen by 2.5-fold to 2.1 million new cases worldwide from 1990 to 2013. The number of cumulative deaths from these malignancies increased by 1.6-fold in the same timespan. The contributory biological influence of gender, tobacco use and obesity likely impact on the incidence and outcomes of urologic malignancies.[8]

Key to addressing a rise in incidence of malignancies and associated morbidity and mortality is understanding the evolving burden of disease in a maturing population when considering the differential outcomes in resource-limited settings. Prioritized research for these prevalent cancers is essential in unlocking complexities that may facilitate targeted health care policies. The 8 published manuscripts and 11 abstracts and presentations (Section 1) comprising this body of work demonstrate the implementation of novel translational medicine techniques to gain further insight into several common urologic cancers.

Consideration must be taken to address morbidity of definitive treatment approaches in the management of uro-oncologic cancers. The risk of any procedure should not outweigh the potential benefit nor impact on quality of life to such a degree that would render it a justifiable liability. The consideration of cancer surveillance in small renal masses and certain localised prostate cancers has revolutionised our approach and has allowed patients to continue their daily lives unchanged. Nonetheless the psychological burden of untreated disease may propel some patients to desire a more definitive approach to the management of their disease. Thus the need for organ conservation, which aids in preserving function and quality of life, has led to the emergence of minimally invasive treatment modalities.

## 2.1 Minimally invasive treatment modalities

Current onco-ablative techniques, including both the most established as well as novel minimally-invasive, focal techniques, include:

**Cryoablation** which involves freezing the renal tumor to less than 20°C using liquid argon or nitrogen, followed by a thawing cycle, performed as a single or double freeze-thaw cycle, which causes tissue denaturation and destruction. Cryotherapy disrupts the cell membrane stability and protein synthesis, and causes direct cellular injury by formation of ice crystals which injure the intracellular structures.[9,10]

**Radiofrequency ablation** which occurs by the transfer of high-frequency electrical current into target tissue culminating in thermal energy. Temperatures in excess of 60°C cause tissue destruction through coagulative necrosis, fibrosis, and thermally induced vascular thrombosis. The conductive heat spreads to adjacent tissue, leading to tissue ablation.[10,11,12]

Similarly **high intensity focused ultrasound** (HIFU) coagulative necrosis of treated tissue (most commonly prostate) is achieved by thermal ultrasound energy.[13]

A recent study compared partial nephrectomy (PN) to RFA and cryoablation for renal lesions ≤ 7cm in 1803 patients (1057 PN, 180 RFA, 187 cryoablation), found that for lesions less than 4cm, local recurrence-free survival was similar between all three techniques, however, metastasis-free survival was lower in the RFA group. Those patients who underwent PN for sub 4cm lesions had a statistically significantly higher overall survival when compared with ablative therapy (PN 95%, RFA 82%, cryoablation 88%). For lesions 4-7cm, PN and cryoablation again had similar local recurrence and metastasis-free survivals, however, PN offered significantly higher overall survival for these patients (PN 93%, cryoablation 74%).[14] It is however important to emphasize that the higher overall survival in the PN group is reflective of a younger, less comorbid population with a better preoperative renal function.

Comparative efficacy of focal therapy in treating clinically significant non-metastatic prostate cancer was published, demonstrating failure free survival was 99% at 1 yr and 88% by year five. For the entire cohort, the oncological outcome was reflective of a metastasis-free, cancer-specific, and overall survival at 5 years was 98% (95% CI 97–99%), 100%, and 99% (95% CI 97–100%), respectively. Functionally, patients

achieved complete pad-free urinary continence and none required more than 1 pad/day.[15]

Current trials investigating innovative technologies have emerged as is the case with **vascular targeted photodynamic therapy** (VTP) for the management of localised prostate cancer. This phase II trial induces focal ablation of tumor lesions through cell necrosis by damaging the tumor vasculature. VTP destroys targeted tissues using a photosensitizer (TOOKAD Soluble [WST11], STEBA Biotech) in association with a low-power near-infrared laser in the presence of oxygen. WST-11 absorbs light and transfers energy to oxygen molecules creating reactive oxygen species inducing local vascular occlusion and cell destruction. TOOKAD is applied intravenously and the illumination of the targeted area by transperineal optical fibers inserted under trans-rectal ultrasound guidance under general anesthesia.[16,17] After a 3.5y standard care follow-up, successful focal ablation was documented for 75% i.e. 51/68 patients remained cancer-free in the treated lobe. The most common low grade side effects included erectile dysfunction (ED; n = 28), lower urinary tract symptoms (n = 14), and perineal pain (n = 9).[18] Despite demonstrating potential clinical application, TOOKAD lacks FDA approval to date.

Novel therapeutic **drug delivery devices** have also been considered for the focal management of localised oncological disease. The device acts as a reservoir for the designated drug as well as the delivery apparatus to provide controlled delivery of the drug to the affected area and hence reduce toxicity and the associated adverse events of systemic treatment.[19] Examples include passive drug delivery implants that release drugs at predetermined rates by osmotic pressure [19,20], a porous membrane[21], polymer degradation[22], or a change in their surroundings such as pH or temperature variation[22,23] with very limited or no dosing control i.e. over the rate and time of release of the drug. Alternatively a major benefit of an on-demand drug delivery apparatus is that the drug release can be switched on and off to suit a proposed treatment regimen but may later be adjusted if a relevant change in dosing is required as a result of an unexpected change in the condition of the patient.[24] Ideally an implanted device delivers the drug on-demand, regulated by an on/off mechanism while maintaining drug stability within the reservoir to enable individually tailored treatment regimes. Microelectromechanical systems (MEMS) have been

used to accurately regulate the dosage and time of drug release, however these mechanisms require a power source (batteries) with which to release the loaded drug. To address this limitation On-Demand™ therapeutics, for example, has developed a laser activated intravitreal drug reservoir for the treatment of ocular diseases[20], magnetic actuation may offer another alternative for these devices. In a study comprising this thesis we hint at the capability of implanting a magnetically actuated drug delivery device, harboring docetaxel, into an animal model for treatment of localized prostate cancer. [20,25]

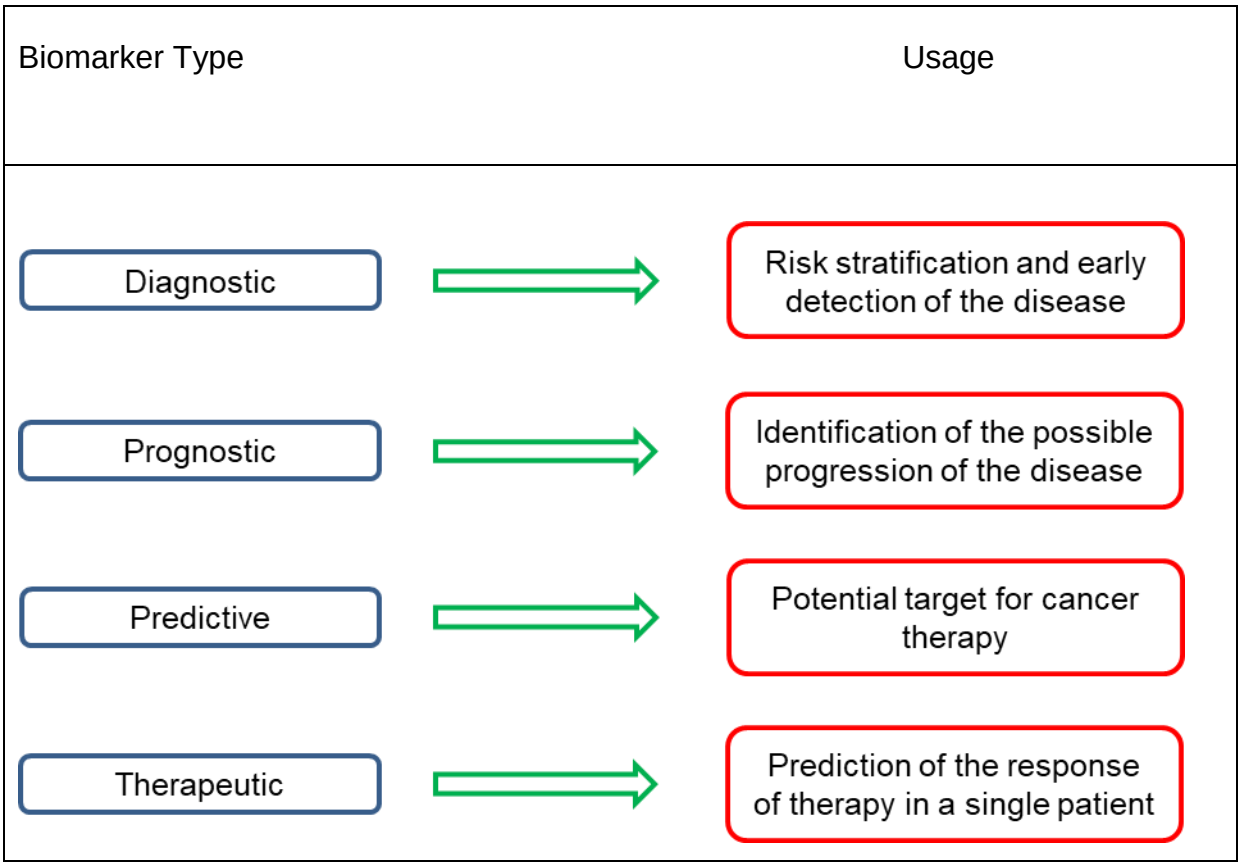
Results were in favour of the device: immunohistochemistry staining evaluating the extent of leukocyte infiltration exhibiting good biocompatibility and oncological efficacy as a sign of chronic inflammation demonstrated the device and the control subgroup displayed similar compatibility. Most affected by signs of chronic inflammation were the mice receiving subcutaneous docetaxel depot injections. Further histopathological interrogation of the device cohort comparatively demonstrated superior efficacy of cellular apoptosis and lack of cell proliferation than any other cohort. Oncologically, our device established comparable tumor growth rate suppression to subcutaneous docetaxel administration and only slightly inferior to intravenous docetaxel application with significantly less adverse events than either subcutaneous or intravenous treatment modalities. Multiple factors contribute to a decision regarding treatment options for patients with localised cancer, including patient age, comorbidities, extent and nature of the disease as well as functional and oncological outcomes. Magnetically actuated or microelectromechanical systems show promise in addressing those concerns well. This study in particular reflects upon the additional options novel clinical treatment approaches may offer for localized (prostate) cancer management in the future.

### 2.2 Biomarkers to better characterise and treat urologic cancers

Prognostic and predictive biomarkers have become a rapidly progressive branch of translational oncology in recent years. Comparatively, prognostic biomarkers provide information about the patient's overall cancer outcome, regardless of therapy. A common prognostic biomarker in uro-oncology is the prostate specific antigen (PSA) for prostate cancer. The predictive biomarker on the other hand not only provides

information about the efficacy of a therapeutic intervention, it also offers a potential target for novel agents. In addition, predictive biomarkers may deliver insight into potential high risk patients. This may equip physicians with improved tools for selecting treatments for individual patients.

Figure 2: Biomarker applicability[26]




In a clinical study encompassed in this manuscript we aimed to explore the pathogenesis of acute, peri-chemotherapeutic vascular events (including arterial occlusions, myocardial infarctions, and cerebral strokes) by investigating potential laboratory parameters associated with vascular disease in young healthy testicular germ cell cancer patients undergoing platinum-based chemotherapy.[27] Comparatively late onset cardiovascular risk including atherosclerotic disease, coronary artery disease, and myocardial infarction is well established in germ cell cancer survivors post platinum-based chemotherapy.[28] The central finding in our study is the increase of von Willebrand Factor (vWF) during the course of cisplatin-based chemotherapy and the subsequent normalization of this value within several

months after completion of treatment. This increase of vWF would suggest endothelial damage secondary to the chemotherapy with consecutive hyperactive coagulation to be the clue to the pathogenesis of associative acute vascular toxicity. The results do not conclusively predict for cardiovascular events, however, remind physicians to remain vigilant when administering platinum-based chemotherapy in this subset of oncological patients.

Given that individual oncological diseases are heterogeneous in their molecular makeup and treatment responsiveness, this often results in the treatment of many patients with ineffective drugs, incurring of substantial medical costs for the treatment of patients who do not benefit and the conducting of large clinical trials to identify small, average treatment benefits for heterogeneous groups of patients. This may delay effective treatment in many cases. In oncology, new genomic technologies provide powerful tools for the selection of patients who require systemic treatment and are most (or least) likely to benefit from a molecularly targeted therapeutic.[29]

Commonly these biomarker investigations are either tissue- or blood-based.
Tissue samples for molecular genetic studies may be available in two main formats, either as fresh frozen samples or as formalin-fixed, paraffin embedded (FFPE) tissue.
Tissue based investigations interrogate three types of macromolecule:
**DNA** - despite a relative resistance to degradation, may be less instructive in a FFPE setting as formaldehyde fragments DNA, resulting in shorter, less informative fragments wherein mutations might be missed or conversely misinterpreted.
**RNA** - is the most labile class of molecule. Functionally RNA generates molecular signatures dependent on the effect of the environment on tumor tissue. For example, RNA signatures of warm ischemia (the length of time devascularized tissue is at body temperature), cold ischemia (the length of time tissue sits at ambient temperature until stabilization by freezing or fixation) or even pre-operative diet (low vs. high protein content) have been generated, possibly influencing assessment of findings.
**Proteins** - remain one of the most widely used biomarker assays in immunohistochemical localization and assessment of expression levels.[30]

Table 1: Potential prognostic or predictive biomarkers in prostate cancer.[31]

| **Biomarker** | **Source** | **Clinical relevance** | **Prognostic *versus* Predictive** |
|---|---|---|---|
| Metastatic status | Clinical | Number of bone metastasis (EOD), visceral metastasis | Prognostic/Predictive |
| Performance status | Clinical | ECOG performance status (0–4) | Prognostic/Predictive |
| Time to CRPC | Clinical | Time from ADT to CRPC | Predictive |
| Prior treatment | Clinical | Number of antiandrogens or steroid | Predictive |
| PSA | Blood | Protein specifically extracted from prostate gland | Prognostic |
| PSA kinetics | Blood | PSA decrease rate under treatment | Prognostic |
| Gleason score | Tissue | Pathological features strongly correlated prognosis | Prognostic/Predictive |
| Lactate dehydrogenase | Blood | Elevated by injuries and various disease including cancer | Prognostic/Predictive |
| Alkaline phosphatase | Blood | Elevated by cancer | Prognostic |

| | | | |
|---|---|---|---|
| | | spreading to bones or liver | |
| Albumin | Blood | An index of nutritional status | Prognostic |
| Hemoglobin | Blood | Decreased by anemia | Prognostic/Predictive |
| Neutrophil-lymphocyte ratio (NLR) | Blood | Elevated NLR predicted poorer OS in various cancer patients | Prognostic |
| Testosterone | Blood | Ligand of AR associating prostate cancer proliferation | Prognostic/Predictive |
| Number of circulating tumor cells (CTCs) | Blood | Increased number of CTCs associating with worse cancer prognosis | Prognostic |
| Androgen Receptor splice variants in CTC (esp. AR-V7) | Blood | Correlating with poor response to ENZA and ABI but good response to Chemo | Predictive |
| Concentration of cell- free DNA (cfDNA) | Blood | Increased abundance of cfDNA associating with worse cancer prognosis | Prognostic |
| AR mutation and copy number in cfDNA | Blood | Correlating with worse efficacy of ENZA and ABI | Predictive |
| Somatic DNA repair mutations | Tissue | Correlating with poor response to ADT, but good response to PARP | Prognostic/Predictive |

| | | | |
|---|---|---|---|
| | | inhibitors | |
| Tumor mutational burden (TMB) | Blood | Elevated TMB is association with response to Immuno-Oncology | Predictive |

ABI, abiraterone; ADT, androgen-deprivation therapy; AR, androgen receptor; cfDNA, cell-free DNA; CRPC, castration-resistant prostate cancer; CTC, circulating tumor cell; ECOG, Eastern Cooperative Oncology Group; EOD, extent of disease; ENZA, enzalutamide; mets, metastases; NLR, neutrophil-lymphocyte ratio; OS, overall survival; PARP, poly-ADP ribose polymerase; Pred, predictive marker, Prog, prognostic marker; PSA, prostate-specific antigen.

Depending on which molecular analysis is deployed (DNA, RNA, proteins), frozen tissue samples are ordinarily preferred (however harder to come by as this requires a multidisciplinary approach to ensure timely preparation) over FFPE. In particular this is the case for RNA and protein analysis as FFPE samples often contain degraded RNA and non-native configurations of phosphorylated proteins.

Given how heterogeneity of pre-analytical variables may impact on the results obtained, including analyte stability (which is different for different analytes i.e. DNA, RNA or protein), a period of warm and of cold ischemia, fixation time, tissue processing, sample storage time and storage conditions, as well as the invasiveness of real time tumor biopsies with associated complications are inevitable. In addition, a single biopsy sample may not represent the full tumor load's heterogeneity.[32,33,34] The urgency for minimally invasive investigations with similar detail was apparent.

The development and rapid expansion of liquid biopsy in translational oncology have addressed this need. There are several sources of tumor material that can be assessed by liquid biopsy: cell-free or complexed nucleic acids including circulating cell-free DNA (cfDNA), of which a subset represent circulating tumor DNA (ctDNA), cell-free RNA (cfRNA), and circulating tumor cells (CTCs). CTCs represent intact, viable non-hematological cells with malignant features that can be isolated from blood.[35] cfDNA is composed of small fragments of DNA that are not associated with cells or cell fragments, originating from apoptotic and necrotic tumor cells but also

from normal cells that are released into the bloodstream.[36] Large scale ctDNA analysis of progressive cancer in view of cataloguing genetic mutations was first established in 2005 by The Cancer Genome Atlas (TCGA), supervised by the National Cancer Institute's Center for Cancer Genomics and the National Human Genome Research Institute.[37] To date 33 cancers have been investigated.

Interestingly, tumors containing ~50 million malignant cells release sufficient DNA for the detection of ctDNA in blood.[38] In contrast, positron emission tomography—computed tomography imaging generally detects tumors measuring no less than 7 to 10 mm in size and containing ~1 billion cells.[39] The liquid biopsy approach holds clear advantages over tissue biopsies: it is a source of fresh tumor-derived material, unhampered by preservatives. It gives real-time assessment of the oncological disease status and sampling blood is minimally invasive, avoiding the complications of tissue-based approaches.[40] Nonetheless a clear limitation of liquid biopsy is the amount of ctDNA shed may be lower than the detectable threshold. Previous studies have shown that ctDNA levels are dependent on location, size, and vascularity of the tumor and therefore lead to a difference in ctDNA levels among patients with similar disease.[41,42] ctDNA is calculated as a percentage of the cell free DNA available and referred to as a fraction thereof.

In this field of translational oncology, initial liquid biopsy studies focused on progressive, treatment-resistant metastatic disease. The aim was to ensure sufficient fractions of detectable ctDNA would be available for analysis. Multiple studies comparatively appraised liquid versus tissue biopsy aiming to establish correlative value or, ideally, to demonstrate the superiority of liquid biopsy. In fact a study investigating the concordance of circulating tumor DNA and matched metastatic tissue biopsy in prostate cancer identified somatic mutations and copy number changes that highly correlated with each other in the matched samples.[43] Interestingly in that study ctDNA sequencing revealed robust changes not present in the paired solid biopsy, including clinically relevant alterations in the androgen receptor and PIK3 pathways, similarly a liquid versus tissue biopsy study of metastatic gastrointestinal cancers showed the interrogation of the liquid biopsy samples, revealed clinically relevant resistance alterations and multiple resistance mechanisms not found in the matched tumor biopsy in 78% of cases.[44] These

studies demonstrate the utility of liquid biopsy as an informative tool to assess the real time genomic landscape of metastatic cancer. The benefit of liquid biopsy lies in its minimal-invasive nature when compared to tissue-based investigations with similar genomic detail.

To ensure an accurate read of the mutations of interest expected to be detected in a ctDNA analysis of a specific cancer, the **coverage** or **depth** of sequencing is important. Coverage/depth is defined as the number of unique reads that include a given nucleotide in the reconstructed sequence being investigated, thereby the actual empirical per-base coverage represents the exact number of times that a base in the reference is covered by a high-quality aligned read.[45] **Deep sequencing** refers to the general concept of aiming for a high number of unique reads of each region of a sequence.[46] The rationale for deep sequencing is, even though the sequencing accuracy for each individual nucleotide is high, the very large number of nucleotides in the genome means that if an individual genome is only sequenced once, there will be a significant number of sequencing errors. Furthermore, many positions in a genome contain rare **single-nucleotide polymorphisms** (SNPs). Hence to distinguish between sequencing errors and true SNPs, it is necessary to increase the sequencing accuracy even further by sequencing individual genomes a large number of times. We were able to demonstrate this principle using a **targeted sequencing** strategy capturing all exons of 72 metastatic castrate-resistant prostate cancer driver genes.[47,48] We have previously demonstrated that this approach identifies over 90% of somatic mutations present in matched metastatic tissue in patients with ctDNA above 2%.[43] More recently studies have shown that even lower fractions of ctDNA can be detected with relative accuracy.[49,50] This immensely broadens the applicability of liquid biopsy and in particular ctDNA analysis as a stand-alone approach to interrogate the genomic landscape of (urologic) cancer.

### 2.3 Cell models in translational oncology

Although recent advances in genomic translational oncology have opened novel avenues of research, there is still a need for preclinical, bench-based cancer research. In particular, the peculiar capability of human cancer-derived cell lines to provide an indefinite source of biological material for experimental purposes has

reinvigorated efforts to exploit these lines for the distinct purpose of capturing geno- and phenotypic cancer biology and to test the therapeutic efficacy of anticancer agents.[51,52] This is fundamentally demonstrated in a publication listed in this thesis in which sunitinib-conditioned renal cell carcinoma cell lines were interrogated for mechanisms of resistance.[53] The study demonstrated high upregulation of Netrin-1 with associative cellular and endothelial migration. Unfortunately silencing of Netrin-1 did not resolve migratory tendencies of the resistant cells as was expected. The lack of clear conclusions is likely in part due to the cellular communicative complexities contributing to metastatic disease progression in treatment resistant disease which are not well represented by this two dimensional cellular model. Evidence shows that conventional 2D conditions (the extracellular matrix components, cell-to-cell and cell-to-matrix interactions) that are important for differentiation, proliferation and cellular functions *in vivo* are lost.[54]

3D cell culture systems are an evolutionary step forward as they exhibit these features that better reflect complex *in vivo* conditions.[55] Cancer research involving studies on biomarkers, invasion, metastasis and tumor angiogenesis have been widely carried out with three dimensional models.[56,57,58]

Provided with the challenges of preclinical research, the emergence of cancer mouse models is of utmost importance as they aim to explore the causal link between candidate cancer genes and carcinogenesis as well as to provide models to develop and test novel therapeutics in depth. Traditionally, immunocompetent and immunodeficient mice with syngeneic and xenograft tumors transplanted subcutaneously or orthotopically have been used.[59] We demonstrated the use of subcutaneously xenografted PC3 cells in our mouse model investigating the biocompatibility and therapeutic efficacy of a magnetically actuated drug delivery device.[25] Patient-derived xenografts (PDX) allow for individualised evaluation of the cancer being investigated. The PDX can serve as the foundation for a program of evidence-based personal oncology by facilitating the determination of the driving alterations before administering therapy to patients, i.e. PDX-bearing mice are treated with applicable drugs, and antineoplastic effects are measured. Only if a clear response is observed, is the agent administered also to the patient from whom the PDX was derived.

## 2.4 Prognosticators for overall survival in urologic cancer

Despite the potential genomic profiling offers in terms of detailed insights into oncological disorders and the application of this knowledge toward targeted therapeutics, the appreciation for objective assessment of the patient outcomes within the setting of clinical trials is crucial to further treatment guidelines and standardisation of care for our patients. Driving the discovery of surrogate predictive markers in clinical trials will allow for timely outcomes and cost effective evaluation of results. Our retrospective analysis aimed to establish an adequate prognosticator for overall survival of prostate cancer patients that developed metastasis in the course of their disease and had progressed on to castrate resistant prostate cancer (CRPC).[60] The study evaluated the impact of the time of occurrence of metastasis on overall survival. Three subgroups were formed for evaluation:

1. Presentation with metastasis within three months of initial diagnosis (de-novo-M);
2. patients developing primary metastasis more than 6 months prior to castration resistance (CSPC-M)
3. patients with newly diagnosed metastasis within 6 months of becoming castration resistant or thereafter (CRPC-M).

Crucially *de novo* metastatic patients progressed to CRPC significantly earlier from diagnosis (median 1.85 years) than the other two groups, CSPC-M (median 7.27 years) and CRPC-M (median 9.15 years), respectively. It is worth noting, however, that CRPC-M had the shortest median overall survival from the date of diagnosis of their metastases (2.94 years) compared to de-novo-M patients (6.41 years) and CSPC-M (7.01 years). Reflecting upon the results, relevant limitations include the lack of uniform follow up, the implementation of diverse imaging modalities to assess disease progression and variations in systemic oncological management. Despite these shortcomings, this study demonstrates time from diagnosis to CRPC (all patients) and time to metastasis (for CRPC-M and CSPC-M patients) are significant prognosticators of overall survival and are therefore valid surrogates in a study setting. Importantly there is no difference in survival between the groups once castration resistance was reached.

In fact, major trials such as SPARTAN [61] (evaluating apalutamide (ARN-509) in men with Non-Metastatic Castration-Resistant Prostate Cancer) and PROSPER [62] (a phase 3 study of enzalutamide in non-metastatic (M0) castration-resistant prostate cancer (CRPC) patients have already enrolled time to metastasis as a clinical endpoint.

# Chapter 3: Clinical application of translational tools in uro-oncology

## 3.1 Suitability of translational oncology in clinical practice

Personalized therapeutics are already being implemented in oncology. Therapy selection based on the genomic characterization of individual tumors is demonstrated across several cancers. Treatment of women with breast cancer is often based on estrogen receptor status (*HER2* amplification status and gene-expression profiles) indicating the prognostic aggressiveness of the disease, with good response to targeted therapies when compared to conventional hormonal strategies.[63] Similarly, the identification of BRAF mutations in melanoma have led to the introduction of BRAF- and MEK-targeted inhibitors with substantial benefit.[64,65] Equally in a phase II study Olaparib demonstrated favourable efficacy in patients with platinum-sensitive relapsed ovarian cancer with underlying BRCA 2 mutations.[66] In particular, BRCA1 and 2 mutations have been identified in familial breast, ovarian and prostate cancers with considerable clinical impact, encouraging the need for genetic counseling in suspected cases.[67] Ultimately persons who are shown to have cancer-predisposition mutations in the germline may serve as sentinels for the identification of families at high risk.

Understandably these developments have led to the consideration of screening high risk populations for underlying genetic mutations. In a landmark publication investigating the germline burden of DNA damage repair gene mutations in men with metastatic prostate cancer, 11.8% showed deleterious alterations in those genes.[68] This has clinical relevance as patients with inherited DNA damage repair gene defects have been shown to have associative early onset, clinically aggressive localized prostate cancer with cancer-specific mortality.[69,70] The study by Pritchard *et al* did not discuss the clinical implications of their findings.[70] In a publication enlisted in this thesis we investigated the distribution and clinical impact of DNA damage repair gene defects in a British Columbian population of men with metastatic castrate resistant prostate cancer.[71] 319 consecutive patients from our liquid biopsy program form the cohort for investigation. Germline mutations in DNA damage repair genes were present in 7.5% of cases, with BRCA2 mutations being the most common, at

two thirds of these. Importantly, clinical response to conventional androgen deprivation was poor, with progression to castration resistant disease at a median of 11.8 months compared to 19 months in the wild-type cohort. Once started on traditional first line androgen targeting agents for castration resistance, median time to disease progression was 3.3 months, nearly half of the time reflective to the wild type correlative, suggesting poor response to androgen targeted therapies overall. Interestingly though, once metastatic castrate resistant disease was established the overall survival was similar at 29.7 months versus 34.1 months, respectively. In addition we investigated the presence of loss of heterozygosity of the intact BRCA 2 allele. We were able to demonstrate in all 11 BRCA2 carriers with assessable somatic status (ctDNA fraction > 35%) displayed somatic hemizygous loss of BRCA2 and RB1, and in 10/11 cases the somatic deletion removed the intact BRCA2 allele. Previously coinciding results had been described in the literature.[68,72] Clinical applicability of LOH identification was shown in a recent phase II trial of olaparib for patients with mCRPC after chemotherapy, the median response of patients with DNA repair defects (germline and/or somatic) was 9.8 months[73]; a significant improvement on overall survival for patients with lethal disease. In fact, recently two PARP-Inhibotors (Olaparib and Rucaparib) have been granted FDA approval for implementation in this setting. Ultimately, therefore, it is feasible to contemplate that co-targeting with therapies that exploit defective DNA repair (eg, olaparib and carboplatin) may be a therapeutic approach in combination with androgen receptor-targeted agents for metastatic castrate resistant prostate cancer patients with germline DNA repair mutations. However, this warrants clinical qualification.

Despite progressive efforts to prolong overall survival in metastatic prostate cancer patients, the disease remains lethal. Interestingly, prolonged androgen deprivation therapy of patients with a history of prostate adenocarcinoma has led to transition of the tumor morphology to a small cell-like phenotype in some cases.[74] Evidence from model systems suggests that under the selective pressure of prolonged androgen deprivation therapy or other androgen receptor-directed therapy, androgen receptor-positive adenocarcinoma cells can acquire neuroendocrine features and small cell morphology.[75,76] Notably, rearrangements of the ERG gene to androgen-driven promoters (e.g. TMPRSS2) are detected at approximately the same prevalence in treatment-related small cell prostate cancer as adenocarcinoma of the prostate. The

TMPRSS2- ERG gene fusion arises in the context of active androgen receptor signaling; therefore, while the fusion gene is often no longer expressed in transitioned small cell prostate cancer, it is indicative of an androgen -positive ancestral clone.[77,78] Rarely do these cancers present as *de novo* metastatic disease. It is unknown whether de novo small cell prostate cancer is ancestrally distinct from treatment-related small cell prostate cancer or whether it too arises from adenocarcinoma, albeit in the absence of therapeutic pressure. Encompassed in this thesis is a study investigating the genomic landscape of *de novo* metastatic small cell prostate cancer. Eighteen patients were identified from which tissue based DNA extraction and sequencing of formalin-fixed paraffin-embedded (FFPE) tissues of specimens were interrogated to identify driver genes responsible for this rare prostatic cancer variant and consider possible targetable alterations for novel therapeutic agents.[79] In our study at the DNA and RNA level, *de novo* small cell prostate cancer largely resembled treatment related small cell prostate cancer. Interestingly three novel characteristics were identified:

1. *De novo* small cell prostate cancer did not harbor any androgen receptor gene alterations indicative of a castrate resistant prostate cancer ancestor exposed to androgen deprivation therapy.
2. Our data suggests that patients with prostate adenocarcinoma harboring multiple ‘hits’ to TP53, RB1, PTEN and other tumor suppressors should be considered at risk of small cell prostate cancer transformation, even before exposure to androgen receptor-directed therapy.
3. Surprisingly, nearly a third of our cohort presented deleterious biallelic alterations to homologous recombination repair (HRR) or mismatch repair (MMR) genes (MSH2 and MSH6) genes potentially enabling tumor suppressor loss.

Commonly, given the expression of neuroendocrine markers chromogranin A and synaptophysin in this setting, patients are consequently managed similarly to other pulmonary and extrapulmonary small cell carcinomas, with platinum-based chemotherapy.[80,81] Homologous recombination repair defects are linked to durable responses to platinum chemotherapy in prostate, breast and ovarian cancers,[82,83,84] which seems to be the underlying cause of efficacy. However given the genomic alterations identified in this study targeted treatments may be more applicable in a subset of these patients. The biallelic loss in DNA damage repair genes may offer

response to PARP inhibitors.[85] While mismatch repair gene defects are associated with hypermutation and patient response to immune checkpoint blockade. Although this was a tissue-based study, future translational research should consider implementing liquid biopsy for ctDNA analysis which seems to be abundant in advanced patients with small cell-like histology.[86] Liquid biopsy may offer a comparatively informative, yet minimally invasive approach to tissue-based interrogation, facilitating individualised cancer management for patients in real time.

Recruited within this thesis is a milestone liquid biopsy study investigating the genomic landscape of *de novo* metastatic prostate cancer.[87] Although assessible ctDNA fractions were expected, given the extent of disease at diagnosis. Improvements in deep sequencing to our previous studies was encouraging to note. We were able to perform liquid biopsy sequencing in 52/53 patients in our study (the threshold for ctDNA detection was 0.5%). Comparative tissue was available in 48 cases. The concordance for mutation detection in matched samples was 80%. 35/53 patients had their ctDNA collected before initiation of conventional androgen deprivation therapy. It was determined that the ctDNA fraction available in those patients with androgen deprivation therapy prior to ctDNA collection was significantly lower than in the treatment-naive cohort (59% of patients with a median of 1% versus 74% and 11% respectively), although ctDNA fractions varied strongly among patients (untreated range 0-84% and 0-51% in the treated subgroup). Patients with visceral metastasis present with the highest ctDNA burden. The comparative ctDNA and tissue investigations revealed similar genomic alterations (*TP53*; DNA damage repair gene defects, especially *BRCA 2*; *CDK12*) as in metastatic treatment-resistant disease, albeit without the androgen receptor gene alterations seen in progressive prostate cancer.

Important conclusions from this study are:

1. ctDNA provides additional information to a prostate biopsy in men with *de novo* mCSPC, but androgen deprivation therapy rapidly reduces ctDNA availability
2. Primary tissue and ctDNA share relevant somatic alterations, suggesting that either is suitable for molecular subtyping in de novo mCSPC
3. The optimal approach for biomarker development should utilize both a tissue and liquid biopsy at diagnosis, as neither captures clinically relevant somatic

alterations in all patients given that ctDNA or prostate biopsy alone was insufficient in 19 cases (36%).

Likewise the need to implement minimally invasive, explorative investigations to analyse the genomic landscape of several other urologic cancers is underway.
The genomic evaluation of cancers in the several publications submitted in this thesis demonstrate not only the novelty, but the applicability of ctDNA sequencing across a spectrum of urologic cancers.[71,79,87,88] In the case of metastatic bladder cancer platinum-based chemotherapy has been considered as the gold standard for first-line treatment.[89] However the immunogenic nature of the disease has offered new treatment avenues to exploit. Several trials have led to the approval of immuno-oncogenics to be used in as a cisplatin-alternative, albeit patients are required to have an adequate Programmed Death-Ligand 1 (PD-L1) status [KEYNOTE 361 (ClinicalTrials.gov identifier: NCT02853305), IMvigor130 study (ClinicalTrials.gov identifier: NCT02807636)].

Upregulation of PD-L1 may allow cancers to evade the immune system. Several novel agents either inhibit the antigen PD-L1 or its receptor counterpart PD-1. Despite these advances, liquid biopsy analysis of metastatic bladder cancer is underexplored. We designed a study in which we applied a combination of whole-exome sequencing and targeted sequencing across 50 bladder cancer driver genes to plasma cell-free DNA from 51 patients with aggressive bladder cancer, including 37 with metastatic disease[88]. In order to ensure robust mutation calling, the ctDNA threshold for detection needed to be ≥ 2% of the total cfDNA available. While the majority of patients with metastasis (24/37 patients) were above this threshold, only 14% of patients with localized disease had adequate ctDNA proportions.

Twelve percent of estimable samples had evidence of genome hypermutation. We were able to reveal an aggressive mutational landscape in metastatic bladder cancer with 95% of patients harboring deleterious alterations to tumor suppressor genes (*TP53, RB1*, or *MDM2*) and 70% harbor a mutation or disrupting rearrangement affecting chromatin modifiers such as *ARID1A.* Interestingly the team I was a part of identified targetable alterations in MAPK/ERK or PI3K/AKT/mTOR pathways, offering administration of novel drug agents.[90,91,92] The study also identified a novel FGFR3-

ADD1 fusion not previously described. Important therapeutic conclusions that can be drawn from implementing ctDNA analysis in this setting are:

1. We identified TSC1/TSC2 alterations which are reported to confer sensitivity to the mTOR inhibitor everolimus
2. Hypermutation in bladder cancer is amenable to checkpoint inhibitors i.e. PD-1 and PD-L1 inhibitors
3. Aberrant PI3K/AKT/mTOR pathway alterations seen in a large proportion of our patients may suggest opportunities for testing of AKT inhibitors such as MK2206 that has previously demonstrated activity in BCa cell lines[93]
4. Mutations in DNA repair genes raise the possibility that ctDNA profiling may also help guide implementation of cisplatin-based chemotherapy more precisely

There is certainly mounting evidence that tumor subtypes and distinct somatic alterations can influence patient response to existing and novel therapies in advanced bladder cancer. As the arsenal of targeted and immunotherapies continues to grow, it is imperative that biomarker development keeps pace to guide their implementation. Our results show that analysis of circulating tumor DNA from the plasma of patients with metastatic BCa captures the somatic mutational profile in most patients. ctDNA profiling can form a practical tool for real-time patient stratification, and thereby avoid the need for tissue biopsy or interrogation of potentially less relevant archival tissue.

Finally, it is important to highlight we observed high total cfDNA yields in patients receiving chemotherapy, in both the localized and metastatic setting, however this was not accompanied by increased (or any) circulating tumor DNA in most instances, suggesting elevated non-malignant cell death in the wake of systemic cytotoxic therapy. Therefore careful consideration should be given to the timing of liquid biopsy analysis. Future studies will need to consider appropriate time intervals for liquid biopsy investigation to maximise potential ctDNA availability.

### 3.2 Augmenting clinical oncological guidelines through translational urology

The proven benefits of personalised oncology in the metastatic setting with the advancement of genomic profiling, including the implementation of minimally

invasive techniques such as liquid biopsy, have generated interest in the assessment of genomic complexities in localised disease as well. Understanding the landscape of localised prostate and bladder cancer, in particular, may offer insights into the nature of the disease with particular consideration for disease progression and disease specific mortality. Ideally the aim would be to identify patients at particular risk of disease recurrence (for bladder cancer) and progression (both bladder and prostate cancer) that warrant more definitive treatment options including surgery or radiotherapy with curative intent. Overtreatment, particularly for patients with non-aggressive prostate cancer, is a particular concern, as major interventional procedures harbor risks and complications that may outweigh the benefit of treatment. Risk stratification is crucial in developing individualised oncological management.

In the case of non-muscle invasive bladder cancer, there are several factors that contribute to the risk of recurrence and progression to muscle invasive disease (which in healthy individuals requires neoadjuvant chemotherapy and cystectomy), including number of tumors, tumor size, rate of recurrence, tumor grade and staging.[94] Follow-up requires regular outpatient clinic appointments involving a cystoscopy under local anaesthetic. This is burdensome on many patients. As some recurrent bladder cancers are not well visualized on white light cystoscopy, urine cytology is sometimes added to aid diagnosis. Cystoscopy advancements that include blue light and narrow band imaging may prove additionally useful. Recent urine-based biomarker testing shows promise. Particularly when interrogating a simple urine sample may be sufficient when discerning recurrent disease. Ideally this may avoid the need for repeat cystoscopy altogether. Phase III-IV biomarkers include:

1. **Nuclear matrix protein 22 (NMP22):** Nuclear matrix proteins are a structural part of the cell nucleus and provide support for the nuclear shape. A member of this family, NMP22, has been found to be elevated in malignant urothelial cells compared to normal urothelium[95]
2. **Bladder tumor antigen (BTA):** Bladder tumor antigen (BTA) tests detect the presence of basement membrane factors in the urine, which are released from tumor cells during stromal invasion[96]

3. **ImmunoCyt/uCyt+:** The ImmunoCyt test is an immunocytological assay based on the microscopic detection of tumor cell antigens by immunofluorescence[96,97]
4. **UroVysion (FISH):** UroVysion is a multicolor fluorescence in situ hybridization (FISH) containing probes to the centromeres of chromosomes 3, 7, 17 and the 9p21 locus (P16 tumor-suppressor gene)[98]

There are several other urine based-tests currently under investigation. Unfortunately none of the tests to date offer a negative predictive value (NPV) together with satisfactory sensitivity and specificity in this setting to be used as a stand-alone option for monitoring disease recurrence (NPV range 74-93%, sensitivity 50-85% and specificity 46-93%).[99] Importantly cases of non-muscle invasive bladder cancer with higher grade disease require BCG instillation therapy to reduce the risk of recurrence and progression.[94,100] In a significant step toward realisation of implementation regarding these non-invasive biomarker tests, the American Urology Association (AUA) guidelines have stated clinicians may use biomarkers to assess the response to intravesical BCG (UroVysion® FISH) and adjudicate equivocal cytology (UroVysion® FISH and ImmunoCyt®).[101]

Localised prostate cancer risk has been stratified using the D'Amico scoring system for more than the last two decades.[102] It is important to note that the classification assesses the risk of biochemical (PSA) recurrence of disease following radical treatment for localised prostate cancer. Importantly though, this risk stratification does not assess prostate cancer specific mortality. When considering offering definitive treatment options to patients, it is important to assess the impact the disease will have on the overall survival of the patient. Overtreatment is a concern, especially when weighing risk and benefit. Two tissue-based genomic tests have been developed to aim to address these concerns.

**Prolaris®** is a 46 gene RNA-based signature to predict prostate cancer specific mortality from tissue biopsy and radical prostatectomy specimens.[103] Prolaris® has demonstrated significant prediction for high risk disease, biochemical recurrence post radical prostatectomy and metastatic progression.[104,105] In addition, two studies surveying clinical decision-making of urologists with the additional results from

Prolaris® to their disposal have led to divergence from the initial arrangements.[106,107] The impact of these altered decisions await evaluation.

The **Decipher** test consists of 22 RNA expression based genomic markers.[108,109] Decipher was independently predictive of metastasis and prostate cancer specific mortality.[110,111] Importantly in the post-prostatectomy setting, Decipher has been evaluated for its ability to inform decisions regarding adjuvant and salvage radiation therapy.[112,113] To determine clinical utility in the post-operative setting, PRO_IMPACT is a multi-institutional prospective study to assess clinical decision-making and patient-reported outcomes after Decipher testing (NCT02080689).

The **OncotypeDx** prostate biopsy test (17 gene panel) calculates a genomic prostate score (GPS) based on genes from 4 different pathways involved in prostate cancer).[108] Unlike Prolaris® and Decipher, OncotypeDx was designed for use with biopsy tissue and does not have a commercially available test for post-prostatectomy risk stratification.[114] Clinical utility studies indicate that OncotypeDx results may also influence decisions about patient management. Badani et al. reported that 18% of recommendations between active surveillance and treatment changed as a result of OncotypeDx and that clinicians also reported the results increased their confidence in decisions.[115]

Despite all tests showing clinical promise, their exact role in localised prostate cancer remains uncertain. The **AUA Guidelines** mentions these biomarkers, however has refrained on making recommendations at this time as to their implementation in clinical practice.

As discussed previously in this chapter with the recent demonstration that men with castration resistant prostate cancer and mutations of DNA damage repair genes (BRCA mutations) respond favorably to PARP inhibitors, there has been great interest in determining who should be tested for mutations and how the presence of mutations should alter therapeutic approach.[71,116] The authors, including our own study encompassed in this thesis, assert that these men might be considered for earlier alternative therapy but it is unclear what therapy that should be and when it should be considered. Certainly in recognition of castration resistance alternative approaches may be warranted. The incidence of inherited DDR gene mutations has led to the **National Comprehensive Cancer Network (NCCN) guidelines**, now recommending germline testing for all men with metastatic prostate cancer.[117] This

recommendation reflects a significant shift in the clinical applicability of translational uro-oncology and cements the need for continued research.

Genomic profiling of urologic cancers has led to clinically significant discoveries which in turn have driven the reconsideration of recommendations regarding clinical practice, as has been partially reflected upon in this thesis. Moreover, despite the fact that research of this nature is costly and time consuming, the need for understanding the genomic complexities of urologic cancers has compelled clinician-scientists to expand clinical trials investigating outcomes of targeted drugs by interrogating the genomics of those participants as well. A study by Annala *et al* demonstrates the application of liquid biopsy in treatment-naive metastatic prostate cancer patients enrolled in a Phase II cross-over trial comparing androgen receptor targeted agents Abiraterone and Enzalutamide.[118] By leveraging plasma specimens collected in this large randomized trial, they are able to report the relative impact common circulating tumor DNA alterations have on patient response to the most widely used therapies for advanced prostate cancer. They were unable to observe evidence for differential efficacy between abiraterone and enzalutamide within any genomic or clinical subgroup, nonetheless the study showed only deleterious alterations in homologous recombination repair genes (*BRCA2* or *ATM*) and *TP53* remained significantly associated with shorter time to progression of disease. Only two patients with mismatch-repair gene mutations were identified with both responding poorly to either targeted agent. Androgen receptor gain was not significantly associated with shorter time to progression after adjustment for ctDNA presence and clinical prognostic factors. It also did not impact on treatment response. However androgen receptor mutations were associated with treatment resistance. Key limitations include the exploratory nature of the analyses (in the absence of a prospective analysis plan) given only 115/202 patients had sufficient ctDNA for genomic evaluation, which prevented the researchers from assessing the clinical relevance of differing combinations of genomic alterations, and whether any specific combinations may be associated with complete lack of benefit from abiraterone or enzalutamide. Nonetheless this pioneering study reiterates the continued need for integration of novel diagnostics techniques with established clinic research to progress our understanding of the cancers we manage and the impact this may have on our patients.

### 3.3 Barriers hampering the clinical implementation of translational uro-oncology

Given the major potential ctDNA analysis offers, application has been found in several subsequent oncological settings as is reflected in the table below.[119]

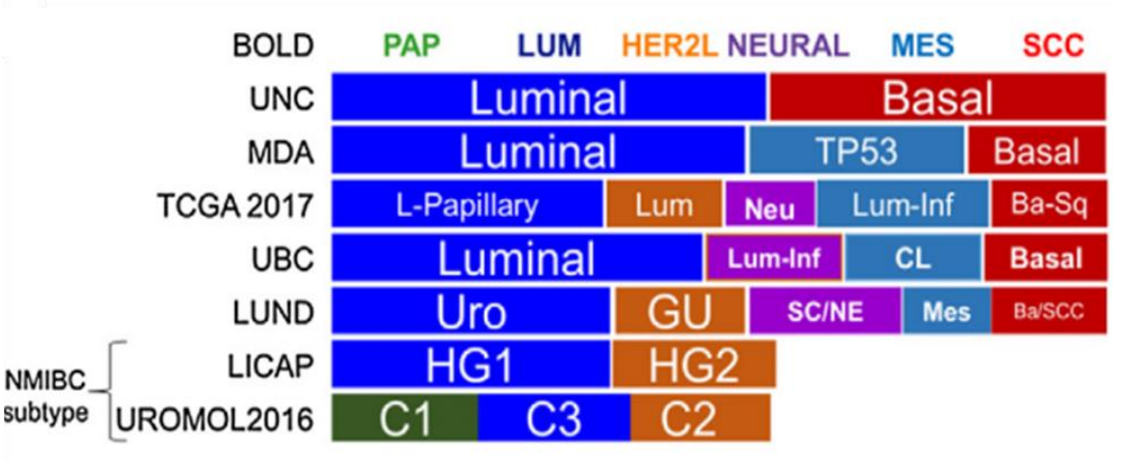
Table 2: Applications of ctDNA in cancer diagnostics[119]

| ctDNA Application | Summary |
|---|---|
| Prognosis determination | • Absence of ctDNA after surgery is associated with a much better prognosis and smaller chances of relapse, nonetheless timing of the liquid biopsy may affect ctDNA availability and thus mask potential recurrent disease<br>• Prognosis determination aids in selecting aggressiveness of treatment as well as determining the necessity for adjuvant therapy; patients at high risk of relapse could receive targeted treatment, while low risk patients are spared unnecessary chemotherapy |
| Monitoring for treatment efficacy/relapse | • ctDNA can be analyzed through a blood test; this 'liquid biopsy' can be repeated more often, enabling consistent monitoring of response to treatment<br>• Raised ctDNA concentrations or increased number of mutations indicate treatment failure/relapse earlier than clinical relapse |
| Selection of treatment | • Sequencing the ctDNA informs choice of therapy to target specific mutations<br>• Traditional tumor biopsies only allow for the analysis of a certain part of the tumor, which ignores intratumor heterogeneity, while ctDNA analysis provides a more holistic view of the tumor to inform more targeted treatment |
| Tumor size/disease burden | • Larger amount of ctDNA in blood correlates with advanced tumor stage/greater metastatic burden<br>• Blood testing does not carry the risk of radiation exposure or poor accuracy of imaging; ctDNA can provide a snapshot of disease burden, which can be repeated more often than imaging or traditional biopsies |
| Detection in asymptomatic individuals | • Most studies show poor sensitivity, especially for early stage tumors<br>• For small tumors, there is not enough ctDNA present to allow for an accurate test result<br>• However, reliable ctDNA tests for early diagnosis would allow for early intervention and curative surgery and higher cure rates |

Despite these advances, many avenues remain entirely unexplored whereas some have partially been investigated. The intricacies of the genomic pathways underlying urologic cancers are plentiful. This was clearly demonstrated by our study investigating the role of netrin-1 in sunitinib-resistant metastatic renal cell carcinoma cell lines. Since the downregulation of netrin-1 has shown to reduce metastasis or tumor growth in multiple cancers, our study sought to investigate whether netrin-1 is also a promising target in mRCC. Unfortunately we did not show a reduction in migration or cell viability in our cell lines after netrin-1 silencing. Hence other factors besides, netrin-1, are contributory to metastatic disease progression and sunitinib resistance in renal cell cancer.[53]

Similarly research into the molecular subclassification of bladder cancer has led to several groups publishing multiple classification variants as reflected by the figure below.

Figure 3: Molecular subtypes of bladder cancer, stratified by various study groups




Color code: Purple = NEURAL; dark blue = LUM; green = PAP; orange = HER2L; red = SCC; light blue = MES. Ba/SCC = basal/squamous-cell carcinoma-like; Ba-Sq = basal-squamous; BOLD = bladder carcinoma subtypes of large meta-cohort database; CL,= claudinlow; diff. = differentiation; ECM = extracellular matrix; GU = genomic unstable; HER2L, HER2-like; LICAP = Leeds Institute of Cancer and Pathology; Lpapillary = luminal-papillary; Lum = luminal; LUM = luminal-like; Lum-inf = luminal infiltrated; LUND = Lund University; MDA = MD Anderson Cancer Center; Mes, mesenchymal; MES = mesenchymal-like; MIBC = muscle-invasive bladder carcinoma; Neu = neuronal; NEURAL = neural-like; NMIBC = nonmuscle-invasive bladder cancer; PAP = papillary-like; SC/NE = small cell/neuroendocrine; TCGA = The Cancer Genome Atlas Network; UBC = University of British Columbia, UNC = University of North Carolina; Uro = urobasal

Understandably a better appreciation for the genomic profile of the disease aids to discern potential high risk cancer with the peril of recurrence and in particular progression. These critical studies have highlighted the complexities of the disease and the difficulty in identifying clear denominators for subclassification. The most recent and thorough investigation to date was published by Tan *el al* (Figure 3).[120] This study identified six bladder cancer subtypes, similar to the TCGA findings. Particularly interesting was that neural-, mesenchymal- and squamous-cell-like subgroups are predominant in progressive bladder cancer involving the muscular layer of the bladder with a comparatively worse overall survival. Although these studies provide insightful information correlative to clinical outcome, it remains difficult to envision clinical application in the near future with clear recommendations by governing clinical bodies as to implementation due to their underlying divergent nature.

Equally our magnetically actuated drug delivery study is another example of progressive advancement of translational medicine in an oncological setting.[25] However the trial presented clear limitations as to the potential applicability in a clinical setting. Despite the fact that biocompatibility and efficacy were proven, obvious constraints of the study were the small cohort sizes (limited by the number of devices), a lack of variable doses of docetaxel in the device and in the comparative treatment arms to more accurately assess treatment efficacy. Moreover, treatment initiation and the tumor measurement time points needed to be adjusted with treatment initiation commencing earlier and the measurements performed at more regular intervals. Finally, the orientation of the aperture of the device toward the tumor and the angle of the actuating magnet are crucial for delivering the optimum dose of docetaxel accurately to the site of interest. Despite these shortcomings the feasibility of a device of this nature was discussed in a review article published in *Nature Reviews.*[121]

### 3.4 Future endeavours of translational uro-oncology

This thesis has demonstrated the manifold capabilities of translational uro-oncology. Several of the studies reflected upon in this manuscript have demonstrated clinical applicability. Others show promise for future successes. The challenge lies in implementing the current knowledge to gain further insight to overcome the limitations of previous investigations.

Liquid biopsy has proven its worth as a minimally invasive technique, applicable in a multitude of circumstances:

1. Interrogating localised cancer by means of tissue and urine based tests to risk stratify patients diagnosed with common urologic cancers
2. Discovering the landscape of treatment-naive advanced cancers to appreciate the genomic profiles of disease
3. Mapping the landscape of advanced oncological disease offering the real time assessment of treatment response
4. Application of genomic profiling in ongoing drug trials to discern non- and low responders better and to offer a more tailored approach to their care.

Future endeavours should aim to continue the progress made to date. Several liquid biopsy studies (especially patented commercially available genomic panels) are cost-intensive and do not allow for routine clinical application. These investigations should be completed in a trial setting. This has been demonstrated by the reiteration of the **AUA guidelines** regarding tissue-based genomic biomarkers for localised prostate cancer. As per the current guidelines, biomarkers have not shown a clear role in the selection of candidates for active surveillance and are not necessary for follow up. These tests are in their infancy, however have drawn the attention of urologists as progressive development and may find use in the future.

Recently, Zhao and collaborators developed and validated the Decipher Post-Operative Radiation Therapy Outcomes Score (PORTOS).[122] PORTOS is a 24 gene predictor of response to postoperative radiotherapy for prostate cancer. The score is not prognostic of metastatic outcome when no radiation therapy is utilized but is highly predictive of metastatic progression if adjuvant or salvage radiation is used, with high PORTOS scores being associated with a 7-fold reduction in metastatic

progression among men receiving postoperative radiation. This exemplifies the progression of development.

At the University of California, Davis, with the Serial Patient Derived Xenograft Models to Eliminate Cancer Therapy Resistance Trial (SPIDER) is underway. This will enable tailored patient stratification strategies, potentially identifying novel predictive biomarkers and can uncover new biology not hypothesized previously. Applicability has been demonstrated by Hidalgo *et al* where targeted treatment was introduced to patients once modulated drug therapy successes were achieved in their corresponding PDX models.[123] The mouse model driven drug delivery device study discussed in this thesis broadly reflects upon its utility.[25]

The examination of potential biomarkers at the RNA, DNA or protein level by interrogating tissue, blood and other bodily fluid is guiding cancer research and clinical oncology increasingly towards molecularly directed therapy. Patient derived xenografts are an important additional tool in translational medicine for drug testing and biomarker development as the PDX provides a model system that recapitulates patient disease with the highest integrity.

The tri-modal applicability of translation oncology in common urologic cancers is well described within this thesis. Progressive benchside research has proven useful in understanding of molecular dynamics in cancer progression. The identification of several novel biomarkers has found bedside utilisation with predictive and prognostic value in guiding oncologic management. In particular, the minimally invasive liquid biopsy analysis has proven to be an invaluable tool to this extent. Both the generalised application of biomarker findings leading to guideline considerations and alterations, as well as the implementation thereof in randomised clinical trials reflects upon the communal component constituting the third pillar of translational medicine.

## Conclusion

This body of work reflects the essence of translational medicine in common urologic cancers. The striving to advance the understanding of cancer dynamics whether in a localised or progressive state aids the identification of biomarkers and actionable targets for implementation in the clinical setting. From the magnetically actuated drug delivery device, offering a novel alternative in the management of localised disease to the minimally invasive liquid biopsy studies interrogating circulating tumor DNA in advanced urologic cancers for genomic alterations that may offer a better understanding of the underlying disease and possible targetable mutations and the search for biomarkers to identify high risk patients susceptible to acute vascular toxicity peri-chemotherapy, this research aims to improve an individualised approach to cancer care. Despite a degree of detail discussed in this manuscript with coverage addressing several fields within translational uro-oncology, truthfully it only offers a mere glimpse into the sheer magnitude of what translational medicine has to offer in our discipline. Ongoing and future translational endeavours strive to investigate more of the intricacies cancer research faces with the shared goal of improving cancer care for our patients.

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## Section 1

| Title | Publication/Poster/Abstract | Journal/Conference |
|---|---|---|
| Magnetically-actuated drug delivery device (MADDD) for minimally invasive treatment of prostate cancer: An in vivo animal pilot study | Publication | 28786159 |
| Circulating Tumor DNA Abundance and Potential Utility in De Novo Metastatic Prostate Cancer | Publication | 30638634 |
| Treatment Outcomes and Tumor Loss of Heterozygosity in Germline DNA Repair-deficient Prostate Cancer | Publication | 28259476 |
| Biallelic tumour suppressor loss and DNA repair defects in de novo small-cell prostate carcinoma | Publication | 30015382 |
| The impact of time to metastasis on overall survival in patients with prostate cancer | Publication | 29488095 |
| Circulating Tumor DNA Reveals Clinically Actionable Somatic Genome of Metastatic Bladder Cancer | Publication | 28760909 |
| Evidence for acute vascular toxicity of cisplatin-based chemotherapy in patients with germ cell tumour | Publication | 22199322 |
| The role of netrin-1 in metastatic renal cell carcinoma treated with sunitinib | Publication | 29854303 |
| Treatment outcomes and tumor loss-of-heterozygosity in germline DNA repair deficient prostate cancer | Abstract | TFI Research Day |
| Clinical and genomic implications of germline DNA repair mutations in metastatic castration-resistant prostate cancer | Abstract | TFI Research Day |
| Circulating tumor DNA prior to therapy initiation in *de novo* metastatic prostate | Abstract | AUA 2018 |

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| cancer | | |
| Dissecting the genomic landscape of metastatic bladder cancer using circulating tumor DNA | Abstract | ASCO-GU 2017 |
| Detection of circulating tumor DNA in de novo metastatic castrate sensitive prostate cancer | Poster | Lorne Sullivan Day 2018 |
| Detection of circulating tumor DNA in *de novo* metastatic castrate sensitive prostate cancer | Poster | ESMO 2018 |
| Germline DNA repair mutations in metastatic castration-resistant prostate cancer: therapy response and applicability of circulating tumor DNA | Abstract | ASCO-GU 2017 |
| Dissecting the genomic landscape of metastatic bladder cancer using circulating tumor DNA | Poster | ASCO-GU 2017 |
| Germline DNA repair mutations in metastatic castration-resistant prostate cancer: therapy response and applicability of circulating tumor DNA | Poster | ASCO-GU 2017 |
| Dissecting the genomic landscape of metastatic bladder cancer using circulating tumor DNA | Abstract | NWUS 2017 |
| Dissecting the genomic landscape in metastatic bladder cancer using circulating tumor DNA | Poster | WSAUA 2017 |

## APPENDIX 1

## Accolades for academic work

2017 Conquer Cancer Foundation Merit Award (GUASCO 2017)
2017 Canadian Urologic Oncology Group Research Trainee Grant Award ($ 10.000)
2017 Best of the Session Poster Winner Western Section AUA 2017

# APPENDIX 2

## Papers included in this submission

### Magnetically-actuated drug delivery device (MADDD) for minimally invasive treatment of prostate cancer: An in vivo animal pilot study

1: Struss WJ, Tan Z, Zachkani P, Moskalev I, Jackson JK, Shademani A, D'Costa NM, Raven PA, Frees S, Chavez-Munoz C, Chiao M, So AI. Magnetically-actuated drug delivery device (MADDD) for minimally invasive treatment of prostate cancer: An in vivo animal pilot study. Prostate. 2017 May;77(13):1356-1365. doi: 10.1002/pros.23395. Epub 2017 Aug 8. PubMed PMID: 28786159.

### Circulating Tumor DNA Abundance and Potential Utility in De Novo Metastatic Prostate Cancer

2: Vandekerkhove G, Struss WJ, Annala M, Kallio HML, Khalaf D, Warner EW, Herberts C, Ritch E, Beja K, Loktionova Y, Hurtado-Coll A, Fazli L, So A, Black PC, Nykter M, Tammela T, Chi KN, Gleave ME, Wyatt AW. Circulating Tumor DNA Abundance and Potential Utility in De Novo Metastatic Prostate Cancer. Eur Urol. 2019 Apr;75(4):667-675. doi: 10.1016/j.eururo.2018.12.042. Epub 2019 Jan 10. PubMed PMID: 30638634.

### Treatment Outcomes and Tumor Loss of Heterozygosity in Germline DNA Repair-deficient Prostate Cancer

3: Annala M, Struss WJ, Warner EW, Beja K, Vandekerkhove G, Wong A, Khalaf D, Seppälä IL, So A, Lo G, Aggarwal R, Small EJ, Nykter M, Gleave ME, Chi KN, Wyatt
AW. Treatment Outcomes and Tumor Loss of Heterozygosity in Germline DNA Repair-deficient Prostate Cancer. Eur Urol. 2017 Jul;72(1):34-42. doi: 10.1016/j.eururo.2017.02.023. Epub 2017 Mar 1. PubMed PMID: 28259476.

### Biallelic tumour suppressor loss and DNA repair defects in de novo small-cell prostate carcinoma

4: Chedgy EC, Vandekerkhove G, Herberts C, Annala M, Donoghue AJ, Sigouros M, Ritch E, Struss W, Konomura S, Liew J, Parimi S, Vergidis J, Hurtado-Coll A, Sboner A, Fazli L, Beltran H, Chi KN, Wyatt AW. Biallelic tumour suppressor loss and DNA repair defects in de novo small-cell prostate carcinoma. J Pathol. 2018 Oct;246(2):244-253. doi: 10.1002/path.5137. Epub 2018 Aug 28. PubMed PMID: 30015382.

### The impact of time to metastasis on overall survival in patients with prostate cancer

5: Frees S, Akamatsu S, Bidnur S, Khalaf D, Chavez-Munoz C, Struss W, Eigl BJ, Gleave M, Chi KN, So A. The impact of time to metastasis on overall survival in patients with prostate cancer. World J Urol. 2018 Jul;36(7):1039-1046. doi: 10.1007/s00345-018-2236-4. Epub 2018 Feb 27. PubMed PMID: 29488095.

**Circulating Tumor DNA Reveals Clinically Actionable Somatic Genome of Metastatic Bladder Cancer**

6: Vandekerkhove G, Todenhöfer T, Annala M, Struss WJ, Wong A, Beja K, Ritch E, Brahmbhatt S, Volik SV, Hennenlotter J, Nykter M, Chi KN, North S, Stenzl A, Collins CC, Eigl BJ, Black PC, Wyatt AW. Circulating Tumor DNA Reveals Clinically Actionable Somatic Genome of Metastatic Bladder Cancer. Clin Cancer Res. 2017 Nov 1;23(21):6487-6497. doi: 10.1158/1078-0432.CCR-17-1140. Epub 2017 Jul 31. PubMed PMID: 28760909.

**Evidence for acute vascular toxicity of cisplatin-based chemotherapy in patients with germ cell tumour**

7: Dieckmann KP, Struss WJ, Budde U. Evidence for acute vascular toxicity of cisplatin-based chemotherapy in patients with germ cell tumour. Anticancer Res. 2011 Dec;31(12):4501-5. PubMed PMID: 22199322.

**The role of netrin-1 in metastatic renal cell carcinoma treated with sunitinib**

8: Frees S, Zhou B, Han KS, Tan Z, Raven P, Wong A, D'Costa N, Fazli L, Struss W, Moskalev I, Chavez-Munoz C, So A. The role of netrin-1 in metastatic renal cell carcinoma treated with sunitinib. Oncotarget. 2018 Apr 27;9(32):22631-22641. doi: 10.18632/oncotarget.25201. eCollection 2018 Apr 27. PubMed PMID: 29854303; PubMed Central PMCID: PMC5978253.

## Abstracts included in this submission

### Presentation at GU-ASCO 2017

Germline DNA repair mutations in metastatic castration-resistant prostate cancer: therapy response and applicability of circulating tumor DNA

Werner J Struss[1], Matti Annala[1,2], Evan W Warner[1], Kevin Beja[1], Gillian Vandekerkhove[1], Amanda Wong[1], Martin E Gleave[1], Kim N Chi[1,3,], Alexander W Wyatt[1]

### Presentation at GU-ASCO 2017 and the Northwestern Urological Society Meeting 2017

Dissecting the genomic landscape of metastatic bladder cancer using circulating tumor DNA

Werner J Struss[1,], Gillian Vandekerkhove[1], Matti Annala[1,2], Tilman Todenhöfer[3], Kevin Beja[1], Amanda Wong[1], Alexander W Wyatt[1], Peter C Black[1]

### Presentation at ESMO 2018

Detection Of Circulating Tumor DNA In De Novo Metastatic Castrate Sensitive Prostate Cancer

W.J. Struss[1,], G. Vandekerkhove[1], M. Annala[1], K.N. Chi[1], M.E. Gleave[1], A. Wyatt[1]

## Oral presentations included in this submission

**ESMO 2018:** Detection of circulating tumor DNA in de novo metastatic castrate sensitive prostate cancer
**AUA 2018:** Circulating tumor DNA prior to therapy initiation in de novo metastatic prostate cancer
**Aurora Biomed Precision Medicine Retreat 2017:** Treatment outcomes and tumor loss-of-heterozygosity in germline DNA repair deficient prostate cancer
**Northwest Urological Society Meeting 2017:** Dissecting the genomic landscape of metastatic bladder cancer using circulating tumor DNA
**Terry Fox BC Node Research Day 2016:** Clinical and genomic implications of germline DNA repair mutations in metastatic castration-resistant prostate cancer
**Northwest Urological Society Meeting 2017:** Dissecting the genomic landscape of metastatic bladder cancer using circulating tumor DNA

## Poster presentations included in this submission

**Genitourinary Cancers Symposium 2017:**
Germline DNA repair mutations in metastatic castration-resistant prostate cancer Therapy response and applicability of circulating tumor DNA Dissecting the genomic landscape of metastatic bladder cancer using circulating tumor DNA

**Western Section AUA 2017:**
Dissecting the genomic landscape in metastatic bladder cancer using circulating tumor DNA

# FORM UPR16

**Research Ethics Review Checklist**

**Please include this completed form as an appendix to your thesis (see the Research Degrees Operational Handbook for more information**

| **Postgraduate Research Student (PGRS) Information** | | | | **Student ID:** | 00948680 |
|---|---|---|---|---|---|
| **PGRS Name:** | Dr. Werner Jan Struss | | | | |
| **Department:** | Faculty of Science and Health | **First Supervisor:** | Dr. John Young | | |
| **Start Date:** (or progression date for Prof Doc students) | **19.06.2019** | | | | |
| **Study Mode and Route:** | Part-time ☒ | MPhil ☐ | MD ☐ | | |
| | Full-time ☐ | PhD ☒ | Professional Doctorate ☐ | | |

| | |
|---|---|
| **Title of Thesis:** | Implication and clinical application of translational medicine in the management of common urologic cancers |
| **Thesis Word Count:** (excluding ancillary data) | 9568 |

If you are unsure about any of the following, please contact the local representative on your Faculty Ethics Committee for advice. Please note that it is your responsibility to follow the University's Ethics Policy and any relevant University, academic or professional guidelines in the conduct of your study

Although the Ethics Committee may have given your study a favourable opinion, the final responsibility for the ethical conduct of this work lies with the researcher(s).

**UKRIO Finished Research Checklist:**
(If you would like to know more about the checklist, please see your Faculty or Departmental Ethics Committee rep or see the online version of the full checklist at: http://www.ukrio.org/what-we-do/code-of-practice-for-research/)

| | |
|---|---|
| a) Have all of your research and findings been reported accurately, honestly and within a reasonable time frame? | YES ☒ NO ☐ |
| b) Have all contributions to knowledge been acknowledged? | YES ☒ NO ☐ |
| c) Have you complied with all agreements relating to intellectual property, publication and authorship? | YES ☒ NO ☐ |
| d) Has your research data been retained in a secure and accessible form and will it remain so for the required duration? | YES ☒ NO ☐ |
| e) Does your research comply with all legal, ethical, and contractual requirements? | YES ☒ NO ☐ |

| **Candidate Statement:** | |
|---|---|
| I have considered the ethical dimensions of the above named research project, and have successfully obtained the necessary ethical approval(s) | |
| **Ethical review number(s) from Faculty Ethics Committee (or from NRES/SCREC):** | previously completed |
| If you have *not* submitted your work for ethical review, and/or you have answered 'No' to one or more of questions a) to e), please explain below why this is so: | |
| Ethical apporvals were completed for the individual studies complied in this PhD by publication thesis. | |
| **Signed *(PGRS)*:**  | **Date:** 9.9.20 |